

Comparative Evaluation of Surface Colonization of Candida on Three Different Denture Base Materials - An In Vivo Study

Tanvi Jaiswal¹, A J Pakhan², S R Godbole³, Seema Sathe⁴, Shruti Bohra⁵, Apurva Deshmukh¹

ABSTRACT

Introduction: Over the years researchers have reported on the frequency and distribution of yeast in the oropharynx of apparently normal individuals and those with systemic or mycotic diseases wearing dentures. This in-vivo study was conducted at the department of Prosthodontics, Sharad Pawar Dental College, Sawangi (Meghe), Wardha to evaluate the growth of candida on three different denture base materials (Heat cure acrylic resin, Chrome cobalt alloy, Flexible denture material velplast) and their subspecies growth in each material. A comparative evaluation was also carried out to identify least growth and sub species among these different denture base materials.

Materials and Method: Three different denture base materials dentures were made and delivered to patients. Swab was taken after 1 month and after 3 months of denture insertion. Samples from rugae area and tissue surface of all three different types of denture materials denture of all patients were inoculated on individual petri dishes containing Sabouraud's Dextrose Agar (SDA, Himedia- India) and Petri dish were separated according to different denture base materials swab. (Group A, Group B, Group C). Culture preparation and growth of Candida Albicans on the specimens was conducted and sub species identification was done using KB006 Hi media subspecies identification kit.

Results: Mean number of colonies for heat cure acrylic material at 1 month was 45 ± 6.86 and at 3 months it was 97 ± 12.02 , Mean number of colonies for Flexible denture material at 1 month was 12.80 ± 4.80 and at 3 months it was 22.50 ± 6.16 , Mean number of colonies for Chrome cobalt base metal alloy at 1 month was 7.00 ± 2.30 and at 3 months it was 13.50 ± 3.02 , along with different subspecies of candida in three different denture base materials.

Conclusion: From our present study, it can be concluded that Chrome cobalt alloy showed less adherence to candidal cells, followed by Flexible denture material (velplast) and last Heat cure acrylic resin. The results were statistically highly significant.

Keywords: Surface colonization of Candida, denture associated stomatitis, Denture base materials.

ture irritation hyperplasia, flabby ridges and oral carcinomas. Denture related stomatitis indicates an inflammatory process of the mucosa that bears a complete or partial dental removable appliance, typically a denture.²

"Denture Stomatitis" is a form of mild chronic erythematous candidiasis. It usually has a predisposition for middle aged or elderly denture wearing individuals as erythema is limited to the area beneath the maxillary denture. Presence of the denture is the only etiologic factor to these situations and not caused by any allergy to the denture material. Denture associated stomatitis is associated with variety of candida.²

"The beginning of the rational systematic of the non- ascosporogenous yeasts". The most important oral yeasts belong to the genus candida. Candida albicans is the predominant yeast species, followed by candida glabrata, candida krusei, candida tropicalis, candida guilliermondii, candida kefyr, and candida parapsilosis³⁻⁵

The human oral cavity is known to harbour multitude of organisms. Amongst them, candida albicans has lately become a cause of great concern to dental professionals. It has been coined as opportunistic pathogen amongst the candida species⁶ since it is the most frequent fungal opportunistic pathogen in humans.

Denture stomatitis is the most common infectious disease affecting the palatal mucosa. The unpolished surface of the denture is a suitable location for candidal proliferation owing to its rough surface. The surface roughness of denture enhances the likelihood of microorganisms to remain on the surface after the prosthesis has been cleaned, thus allowing continuous re-infection of palate.

Denture wearing is a predisposing factor for oral colonization by candida and its prevalence can increase from 60-100%.^{7,8} The microenvironment, formed under the denture, is protected from the washing action of saliva to remove debris and microorganisms.^{9,10}

This is dependent on the initial attachment to the denture impression surface, which in turn depend on the physical

INTRODUCTION

The oral cavity may act as a habitat for several pathogens related to systemic infections.¹ Dental Prosthesis causes an alteration in the oral micro flora. Lesions of the oral mucosa associated with the wearing of dentures may represent acute or chronic reactions to microbial denture plaque, a reaction to constituents of the denture base material, or a mechanical denture injury. These lesions constitute a heterogeneous group with regard to pathogenesis. They include stomatitis caused by dentures, angular cheilitis, traumatic ulcers, den-

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properties of the material surface, such as porosity, surface free energy, hydrophobicity and roughness. These variables are all influenced by the polymerization method, material used, and any surface modifications. For instance, candida adhesion to PMMA- based resin is a common source of oral cavity infection.

Many studies stated that the probability of acidic pH prevailing beneath the maxillary denture may potentiate the palatal inflammation associated with denture stomatitis.¹¹

Therefore this study was carried out to evaluate and compare the least growth of candida and their subspecies in three different denture base material (Heat cure acrylic resin, chrome cobalt alloy, and flexible denture material velplast). Null hypothesis of the study is that there is no difference in time dependent surface colonization of candida in three different denture base materials. Alternative hypothesis would be that there is difference in time dependent surface colonization of candida in three different denture base materials.

MATERIALS AND METHODS

Materials used in the study

Three types of denture base materials:- Heat cure acrylic resin (DPI), Chrome cobalt base metal alloy (Wironit), Flexible denture material (velplast), Sabouraud's Dextrose Agar, KB006 Hi Candida Identification kit, Swab sticks, Colony counter, Inoculating loop, Light microscope, Incubator, Induction casting machine, Acrylizer.

Selection of cases

Study was conducted on different patients wearing removable partial denture made up of three different types of materials i.e. Heat cure acrylic resin denture base material, flexible denture base material(velplast) and Cast partial denture base material, which was delivered to the patients 1 month and 3 months prior to the study.

Exclusion criterias

- Patient should not be a previous denture wearer.
- Patients having Gingival and Periodontal disease.
- Patients suffering from systemic conditions like uncontrolled diabetes, hypertension, hyperthyroidism and other cardiovascular disorders.

Methodology

Comparative study of surface colonization of candida on all the three denture base materials and their culture preparation was conducted in Sharad Pawar Dental College, Sawangi (M), Wardha.

Sample collection was done using oral swab technique. Oral swabs were collected from rugae portion of hard palate of patients and tissue surface of upper denture of all three denture base material's denture according to a 2cm x 2cm template delimiting the area to be swabbed covered by denture. This was done immediately after removing of the denture (Fig 1,2) For each patient, SDA was inoculated and medium was incubated at 37°C for 48 hours. After completion of incubation period, the specimens were removed using sterile

forceps to avoid any contamination.

Growth on SDA within 24-48 hrs at 37°C was identified as candida species. Colony counting was done with the help of colony counter to identify the growth and number of colonies in all the three materials after 1 month and 3 months (Fig 3-5). Staining was done using Gram staining technique. Specimens were washed in water and the stained smear was allowed to dry in air. A drop of cedar-wood oil was placed over the specimen on the glass slide and observed under oil immersion lens (1000x) of microscope.

Microscopically, Gram positive yeast cells are dark purple and show characteristic budding which is considered positive for candida (Fig 6). Germ Tube Test (Raynauld's-Braude Phenomenon) was carried out. This rapid screening procedure was done for observing germ tube production which identifies and differentiates *C. albicans* and *C. dubliniensis* from other candida species. SUB SPECIES IDENTIFICA-



Figure-1: Swab from rugae area



Figure-2: Swab from tissue surface of denture

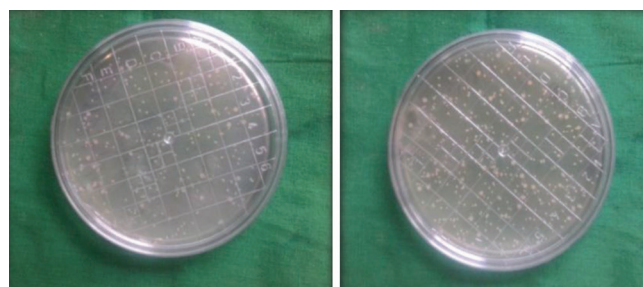


Figure-3: Growth after 1 month swab in Heat cure acrylic material; Growth after 3 month swab in Heat cure acrylic material

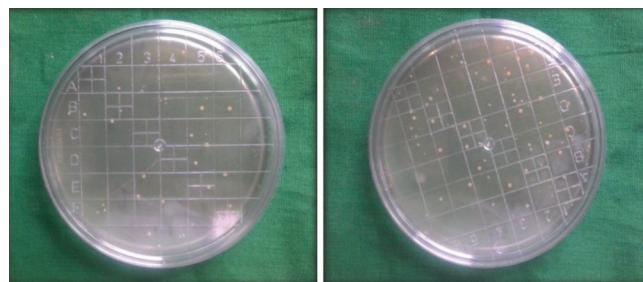


Figure-4: Growth after 1 month swab in Flexible denture material (Velplast); Growth after 3 month swab in Flexible denture material (velplast)

TION was done using KB006 HI Candida™ Identification kit (stored at 2-8°C Shelf life is 12 months). KB006 is a standardized test system that can be used for identification and differentiation of candida species. Each KB006 kit is a standardized calorimetric identification system utilizing twelve conventional biochemical tests. The tests are based on the principle of pH change and substrate utilization. On incubation, organisms undergo metabolic changes which are indicated by a spontaneous colour change in the media. (Fig 7-9)

STATISTICAL ANALYSIS

The statistical tests used for the analysis of the result were: Students paired t test, way ANOVA, Multiple Comparison Tukey Test, Chi square Test. Results were procured with the help of SPSS software version 21.

RESULTS

Mean number of colonies for heat cure acrylic material at 1 month was 45 ± 6.86 and at 3 months it was 97 ± 12.02 . Mean number of colonies for Flexible denture material at 1 month was 12.80 ± 4.80 and at 3 months it was 22.50 ± 6.16 . Mean number of colonies for Chrome cobalt base metal alloy at 1 month was 7.00 ± 2.30 and at 3 months it was 13.50 ± 3.02 .

For heat cure acrylic material:- By using student's paired t-test statistically significant difference was found in mean number of colonies at 1 and 3 months ($t=12.17$, $P\text{-value}=0.000$)

For Flexible denture material:- By using student's paired t-test statistically significant difference was found in mean number of colonies at 1 and 3 months ($t=11.15$, $P\text{-value}=0.000$)

For Chrome cobalt base metal alloy:- By using student's paired t-test statistically significant difference was found in mean number of colonies at 1 and 3 months ($t=9.04$, $P\text{-value}=0.000$) (Table 1, Graph 1)

C. Albicans was present in all 100% patients, C.famata in 40% and C. Glabarata was present in 50% of the patients. By using Chi Square test statistically significant difference was found in three subspecies ($\chi^2=8.90$, $p=0.011$) (Graph 2) C. Albicans was present in all 30% patients, C.Krusei in 70% and C. Glabarata was present in 20% of the patients. By using Chi Square test statistically significant difference was found in three subspecies ($\chi^2=8.14$, $p=0.014$) (Graph 3) C. Albicans was present in all 100% patients, C. Glabarata in 30% and C. Famata was present in 10% of the patients. By using Chi Square test statistically significant difference was found in three subspecies ($\chi^2=17.95$, $p=0.0001$) (Graph 4)

DISCUSSION

Edentulousness is not a disease entity by itself but is a consequence of aging and pathology. The incidences of edentulousness have questioned the adequacy of dental treatments. The treatment objectives of such individuals with artificial prosthesis is not only to restore the function of mastication

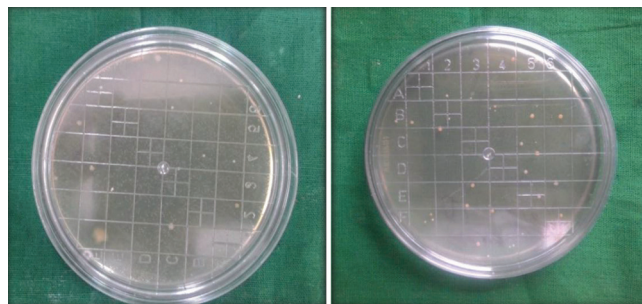


Figure-5: Growth after 1 month swab in Chrome cobalt alloy material; Growth after 3 month swab in Chrome cobalt alloy material

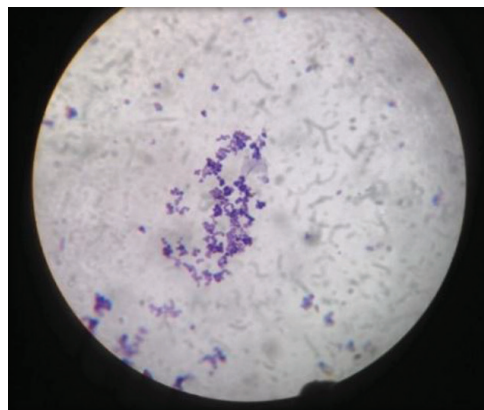


Figure-6: Gram positive dark purple yeast cells with budding, shows positive for candida.



Figure-7 Positive for candida krusei, candida albicans and candida Glabrata in flexible denture material (Velplast)



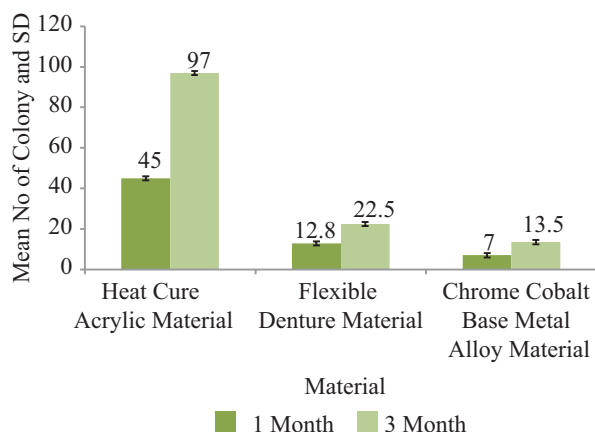
Figure-8: Positive for candida Famata, candida albicans and candida Glabrata in Chrome cobalt alloy material.



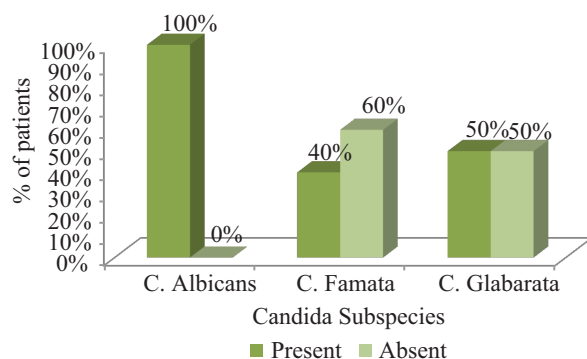
Figure-9: Positive for candida Famata, candida albicans and candida Glabrata in Heat cure acrylic material.

Material	Month	Mean	N	Std. Deviation	Std. Error Mean
Heat Cure Acrylic Material	1 month	45.00	10	6.86	2.17
	3 month	97.00	10	12.02	3.80
Flexible Denture Material	1 month	12.80	10	4.80	1.51
	3 month	22.50	10	6.16	1.95
Chrome Cobalt Base Metal Alloy Material	1 month	7.00	10	2.30	0.73
	3 month	13.50	10	3.02	0.95

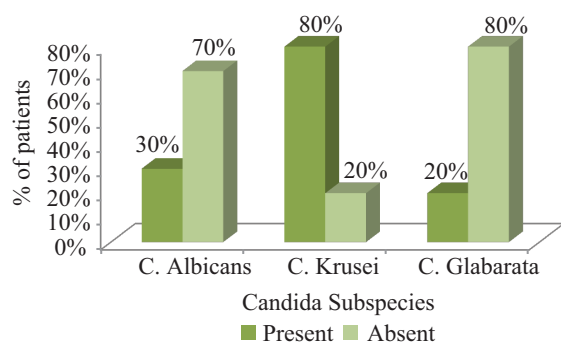
Table-1: Comparison of surface colonization of candida in three materials Descriptive Statistics



Graph-1: Comparison of surface colonization of candida in three materials

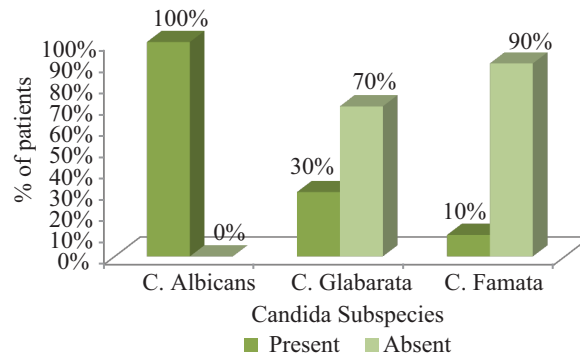


Graph-2: Identification of sub species of candida in heat cure acrylic material



Graph-3: Identification of sub species of candida in flexible denture material

but also to provide acceptable esthetics, improved phonetics and rehabilitation of these patients back to society with elevated level of confidence and comfort.¹ The material used for making the dentures are metallic and



Graph-4: Identification of sub species of candida in Chrome Cobalt Base Metal Alloy Material

non metallic in nature. Majority of complete denture prosthesis are fabricated using acrylic resin due to its good qualities including ease of manipulation and also the cost. The denture bases have three surfaces i.e. tissue surface, polished surfaces and occlusal surfaces. All these surfaces have lots of uneven configuration which will allow food and debris to get accumulated.

Oral conditions particularly associated with wearing of dentures is denture associated stomatitis (DAS) or denture related stomatitis. According to Nikawa et al., the term “denture related stomatitis” would be preferable to “denture induced stomatitis”, since the inflammation of (palatal) mucosa is not induced by the denture, but by wearing the denture or by plaque on the denture.

Candidiasis is caused by infection with species of the genus *Candida*, predominantly with *Candida albicans*. *Candida* species are ubiquitous fungi that represent the most common fungal pathogens that affect humans.

Candidal colonization was seen in 95% of denture wearers, which is in accordance with the study done by Sylvie LA, Jean PG, Noella in 2007. In non denture wearers, 75% showed candidal growth due to poor oral hygiene and poor maintenance of denture, which is consistent with the study done by Daniluk et al in 2006. So this present study was carried by taking candida into consideration than other microbes and their growth variation in denture wearers.¹²

Numerous yeasts are commonly found on the palatal surface of denture and this lends support to the theory that the upper denture act as a reservoir of infection.⁶

Removable denture wearers, especially elderly edentulous patients have poor or low level of oral hygiene practice due to physical health, occupation and social background. Wearing of dentures produces a micro environment conducive to the growth of candida with low oxygen, low pH, and an an-

aerobic environment.

Therefore, aim of our study is to comparatively evaluate the growth of surface adherence of *Candida* to three commonly used denture base materials. The three denture base materials used for this study were Heat cure acrylic resin, Flexible denture material(velplast) and chrome cobalt alloy denture base material. Comparative evaluation of surface colonization after 1 month and after 3 months post insertion of denture shows a significant difference of colonization, found to be less in chrome cobalt alloy as compared to flexible denture material and as compared to heat cure acrylic resin material. In this study we use KB006 HI *Candida*TM Identification kit to identify the type of candidial subspecies which grow in different types of denture base material. No such type of study was carried out before.

The result of our study shows the presence of various types of subspecies in different denture base materials used in this study.

Scope of the present study

There is a scope for further exploration of the materials used for making denture bases which shows least adherence for microbial buildup in relation to oral cavity.

Various subtypes were also identified. These subtypes have been proven to be resistant to various anti-fungal drugs so sub-typing is necessary for the effective management of the disease and to avoid systemic candidemia and disseminated infections which are fatal in nature.

Limitations of the present study

In the present study we have not used molecular method for identification and confirmation of the subtypes of *Candida*. The present study had been carried out with limited number of samples.

CONCLUSION

Denture stomatitis is more frequent in patients with poor denture hygiene. Therefore, the patients should be instructed carefully on denture hygiene and denture cleaning habits. Chrome cobalt alloy denture base material showed less adherence of *Candida* colonies compared to heat cure acrylic resin material where as Flexible denture velplast showed fewer adherences of *Candida* as compared to heat cure acrylic resin material but more as compared to chrome cobalt alloy material.

This study has clearly given us the idea about the important effects of different surface roughness, chemical, physical and hydrophobic properties of different denture base materials on candidal adhesion.

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